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Gene therapy of lysosomal storage disorders

A Salvetti, J M Heard and O Danos

Retrovirus et Transfert Genetique, Institut Pasteur, Paris, France

Lysosomal storage disorders (LSD) result from deficiencies in enzymes normally implicated in the catabolism of macromolecules inside the lysosome. Many of these enzymes can reach the lysosome after being secreted in the extracellular medium and recaptured by specific cell surface receptors. This has suggested a rationale for therapeutic approaches in LSD, in which the missing enzyme is provided by an external source. Current therapies based on this concept, including the administration of purified enzyme and bone marrow transplantation, have been shown to result in clinical improvements in both animal models and patients. Although considerable difficulties must be surmounted, LSD present a favourable situation for gene therapy. The gene corresponding to the affected enzyme has been identified in most diseases and cDNAs are available. Low and unregulated levels of enzyme activity should be sufficient for correction. Importantly, a variety of gene transfer strategies can be carefully evaluated in animal models.

Most of the catabolism in the living cell takes place in the lysosome. These organelles are formed in the trans Golgi network, from vesicles in which more than 40 different enzymes, mostly acid hydrolases, are selectively packed. The mature lysosome results from a fusion between these enzyme-containing vesicles and late endosomes where macromolecules awaiting disposal are entrapped. The acid pH maintained within the lysosome activates the enzymes, and proteins, nucleic acids and complex sugars are degraded. A deficiency in one of these digestion processes results in the accumulation of the undegraded substrate within the lysosomes, which increase in number and size and can severely impair the physiology of the cell.

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More than 30 lysosomal storage disorders (LSD) have been identified which are usually classified according to the undigested macromolecule which accumulates: glycosaminoglycans in **mucopolysaccharidoses** (MPS), sphingolipids in **lipidoses** and glycoproteins in **glycoproteinoses**.[1] Most LSD are due to a failure to synthesize an active form of the relevant enzyme; in some cases, as in the I-cell disease, the defect lies in the inability to target the enzymes to the lysosome (Table). The tissues most affected by the enzyme deficiency are those in which the accumulation of the undigested substrate is the highest. For example, in Krabbe disease the deficiency in galactosylceramidase affects mainly the cells of the central nervous system where the turnover of galactosylceramide is particularly important. In MPS, the missing enzymes are normally implicated in the degradation of glycosaminoglycans which accumulate in spleen, liver, brain and cartilage resulting in bone and joint abnormalities, hepatosplenomegaly, corneal clouding and mental retardation.[2] Similar symptoms are found in patients with Gaucher disease in whom a defect in glucocerebrosidase results in the accumulation of glycosylceramide in monocytes/macrophages.

Many enzymes implicated in LSD are secreted proteins with the notable exception of glucocerebrosidase and acid phosphatase that behave like membrane-associated proteins. In a classic series of experiments, Neufeld and collaborators showed that fibroblasts from MPS patients could be corrected by factors secreted by normal fibroblasts or present in urine concentrates. These 'corrective factors' were identified as the normal enzymes themselves, which were taken up by the mutant cells and targeted to the lysosomes.[1] these enzymes are normally synthesized on membrane-bound polysomes in the rough endoplasmic reticulum and are glycosylated during transit through the endoplasmic reticulum and the Golgi apparatus. There, they are specifically modified by phosphorylation of mannose residues and become ligands for the mannose-6-phosphate receptors (M6PRs). These membrane-anchored receptors cycle between the Golgi compartment, lysosome and the plasma membrane, and direct the phosphorylated enzyme precursors to the organelles, either by selectively packing them into pre-lysosomal vesicles, or by capturing mannose phosphorylated molecules in the extra-cellular environment.[3]

The discovery of this secretion/recapture mechanism has suggested that lysosomal deficiencies could be complemented *in trans* by supplying the missing enzyme either as a purified protein or as a graft of cells secreting the protein. Indeed, in some cases of LSD, treatments involving the infusion of purified enzyme or bone marrow transplantation have demonstrated a therapeutic efficiency in both animal models and patients. The gene corresponding to the affected enzyme has been

Table

Type/syndrome	Enzyme deficiency	Cloned cDNA	Animal models	Affected tissues[a]
MUCOPOLYSACCHARIDOSES				
I/Hurler	α-L-iduronidase	human, canine	dog, cat	CNS, JB, LS
I/Scheie				
II/Hunter	iduronate sulfatase	human	–	CNS, JB, LS
III A/San Filippo A	heparan N-sulfatase	–	–	CNS
III B/San Filippo B	N-acetyl-α-glucosaminidase	–	–	CNS
III C/San Filippo C	acetyl CoA: α-glucosaminide -acetyltransferase	–	–	CNS
III D/San Filippo D	N-acetylglucosamine 6-sulfase	human	goat	CNS
IV A/Morquio A	galactose 6-sulfatase	human	–	JB
IV B/Morquio B	β-galactosidase	human	–	JB
VI/Maroteaux-Lamy	arylsufatase B	human, feline	rat, cat	JB
VII/Sly	β-glucuronidase	human, rat, mouse	mouse, dog	CNS, JB, LS
GLYCORPROTEINOSES				
Fucosidosis	α-L-fucosidase	human	dog	CNS, JB
α-Mannosidosis	α-mannosidase	–	cat, cow	CNS, JB, LS
β-mannosidose	β-mannosidase	–	goat, sheep, cow	CNS
Aspartylglycosaminuria	aspartylglycosaminidase	human	–	CNS, LS
Sialidose	sialidase	–	–	CNS, JB
Galactosialidosis	protective protein	human	dog	CNS, JB, LS

(Table continued on following page)

Table Continued...

Type/syndrome	Enzyme deficiency	Cloned cDNA	Animal models	Affected tissues [a]
LIPIDOSES				
Fabry	α-galactosidase	human	–	kidney
Farber	ceramidase	–	–	JB
Gaucher	glucocerobrosidase	human	mouse	CNS, JB, LS
Krabbe	galactosylceramidase	–	mouse	CNS
GM1 gangliosidosis	β-galactosylceramidase	human	dog, cat	CNS, JB, LS
GM2 gangliosidoses:				
Tay-Sachs	β-hexosaminidase alpha-subunit	human	–	CNS
Sandhoff	β-hexosaminidase, β-subunit	human	cat	CNS
Metachromatic Leukodystrophy	aryl sulfatase A	human	–	CNS
Niemann-Pick A and B	sphingomyelinase	human	–	CNS, LS
Niemann-Pick C	–	–	mouse	CNS, LS
OTHER DISORDERS WITH SINGLE ENZYME DEFECT				
Wolman	acid lipase	–	rat	LS
Pompe	α-glucosidase	?	–	Muscle
I-cell disease and	6-phosopho-N-acetylglucosamine	?	–	CNS, JB

Summary of lysosomal storage disorders. [a]Predominantly affected tissues in the most severe forms are indicate (CNS: central nervous system; JB: Joint and bone; LS: liver and spleen)

identified for most LSD and cDNAs are available (Table). Gene transfer represents an interesting alternative approach for the therapy of LSD. It could be used to provide the enzyme *in trans* or to restore the production of a normal enzyme directly in the affected cells. This review describes possible approaches for gene therapy of LSD and discusses their potential as compared to currently available treatments.

DESCRIPTION OF LYSOSOMAL STORAGE DISORDERS

Genetics

The estimated incidence of LSD is approximately 1:10 000 live births. The most prevalent lysosomal disorder is Gaucher's disease with a significantly higher frequency in Ashkenazi Jew population (approximately 1:600 to 1:2500).[4] The same biased incidence has been found for Tay-Sachs disease which is more frequent in Ashkenazi Jews and the French-Canadian populations.

Most LSD share common clinical features, such as mental retardation and abnormal skeletal development. Many of these disorders also cause hepatosplenomegaly which may be the dominant symptom in the milder forms. Within each type of LSD, different forms can be distinguished on the basis of the severity of symptoms and age of onset. The most severe forms appear early in infancy and the disease has a chronic and progressive course leading to death before adulthood. Milder forms can lead to late onset symptoms that do not cause premature death. A property shared by these disorders is the accumulation of undegraded molecules, which may be excreted in the urine and result at the histological level, in the appearance of cells containing enlarged lysosomes or inclusions. The diagnosis of LSD is usually made on fibroblast or leucocyte extracts, using enzyme assays to identify the deficiency.

The gene encoding the normal enzyme has been identified and cloned in several cases (Table) and molecular studies can identify the most common mutations. Analysis of the mutations found in Gaucher, metachromatic leukodystrophy, GM2 gangliosidoses, aspartylglycosaminuria and several MPS, indicates that these diseases are very heterogenous.[1] In some cases, these genetic studies have established a correlation between the genetic lesions and the severity of the disease. For example, the analysis of several MPS I patients has led to the identification of at least 3 common mutations associated with the development of severe forms.[5–7] In Gaucher disease, over 35 different mutations have been documented including missense and nonsense point mutations, splicing mutations, deletions, insertions and a fusion gene. For some of these mutations a correlation was made with the severity of the disease dependent on whether they provoke a complete or partial lack of the enzyme.[8] However, a certain degree of variability exists among patients bearing the same genotype, impairing the reliability of predictions bout the clinical outcome.

Animal models

The characterization of animal models of LSD (Table 1) makes it possible to evaluate the efficiency of new therapeutic approaches. Small laboratory animals like mouse and rats, which can be easily bred on an homogenous genetic background have been described in the case of MPS VI, MPS VII and Krabbe disease.[9–11] Larger animals, like MPS I, MPS VII and fucosidosis dogs or MPS I and MPS VI cats are useful in preclinical studies designed to evaluate the feasibility and efficiency of a gene transfer protocol on a larger scale.[12–16] Many other animal models of LSD have been described,[17–26] but in most cases the deficiency was only documented at the biochemical level without definitive identification of the genetic defect.

New animal models can also be created in mice by knocking out the relevant gene. This method has been used to engineer a model of Gaucher disease by disrupting the glucocerebrosidase gene. Mice homozygous for this mutation have a very low enzyme activity and die early after birth.[27] Although animal models for milder forms of Gaucher disease have to be created for therapeutic experiments, this first model is important for the investigation of the pathogenesis of the most severe forms of this disease.

CURRENT TREATMENTS

Preclinical studies on animal models

Studies on animal models of LSD mainly consist in enzyme replacement therapy through bone marrow transplantation. The rationale for this approach is that the lysosomal enzyme secreted by the engrafted hematopoietic cells will be distributed to different tissues and taken up by deficient cells. In addition, non-deficient cells differentiating toward the monocyte/macrophage lineage probably reduce storage in surrounding deficient cells by degrading glycosaminoglycans accumulated in the local environment. Bone marrow transplantation has been shown to have beneficial effects in MPS I dogs, with a decrease in glycosaminoblycans storage in various tissues including the brain, and a much slower progression of the disease. However, only a slight impact on the evolution of the skeletal deformities was observed.[28,29] A clinical amelioration was also demonstrated in MPS VI cats and in fucosidosis dogs.[30,31] In the latter case, a rapid improvement in the peripheral nerve and visceral lesions as well as a more gradual improvement in the central nervous system pathology were documented. Notably, these experiments illustrate that the effectiveness of this treatment depends

on the age at the time of transplantation. Engraftment at an early age, before the onset of clinical signs reduced the severity and slowed the progression of neurological lesions. Similarly, and effect on skeletal deformities and on brain lysosomal storage was observed only after treatment of neonatal MPS VII mice[32] and of one month-old MPS VII dogs (M Haskins, personal communication). A neurological improvement was also demonstrated in the Twitcher mouse which has a galactocerebrosidase deficiency analogous to Krabbe disease in humans. The increase in galactosylceramidase activity in the brain correlated with the progressive infiltration of donor-derived macrophages.[33] These cells may progressively reduce storage lesions through local enzyme release, cell-to-cell enzyme exchange and phagocytosis of the undigested products. On the other hand, bone marrow transplantation has no effect on the progression of the neurological disease in dogs with GM1 gangliosidosis.[34] The reduction of the neurological lesions observed in some of these experiments is thought to result mainly from the colonization of brain by donor-derived macrophages. However, injection of purified recombinant β-glucuronidase in newborn MPS VII mice have suggested that enzyme molecules can cross the blood-brain barrier when the treatment is initiated very early in life.[35]

Treatment of patients with LSD

The discovery that lysosomal storage in cell culture can be reduced by providing extracellular enzymes has rapidly led to several clinical trials in patients using plasma or cells as sources of enzymes. However, these experiments always resulted in a minimal transient effect.[36]

Allogenic bone marrow transplantation has now been performed on a large number of LSD patients. HLA matched bone marrow transplantation is available to less than half of the patients. Mortalities are 10% and 25% depending whether an HLA-matched relative or an unrelated HLA-matched donor can be found. Biochemical and clinical benefits have been observed in MPS I, MPS II, MPS VI and Gaucher type I patients. Successful engraftment always results in increased enzyme levels in leucocytes and normalization of the liver and spleen sizes. In MPS I and II, a stabilization of skeletal lesions usually occurs, but little improvement of pre-existing lesions is seen. In these cases, severe neurological symptoms appear to be prevented by early transplantation, but definitive conclusions about intellectual development cannot be drawn in the absence of long-term follow up. Successful engraftment can also be effective in mild forms of Krabbe, Niemann-Pick A and metachromatic leukodystrophy, but not in severe cases.[37,38]

Early trials of enzyme infusion conducted in the 1970s on patients affected with Fabry and Gaucher diseases were encouraging.[1] The procedures for large-scale purification of lysosomal enzymes have now been further developed, especially for the treatment of Gaucher's disease. Glucocerebrosidase can be concentrated from human placenta and processed by modifying the oligosaccharide chains, thus exposing the mannose residues necessary for recognition and uptake by macrophages.[39] More than 200 Gaucher patients with the non-neuronopathic form of the disease (type I), have received regular injections of this preparation (Ceredase®). Hematologic recovery, reduction of hepatosplenomegaly and skeletal improvement have been documented.[40,41]

Enzyme therapy could be applied in many other forms of LSD at least as a transient therapy while awaiting a suitable bone marrow donor. However, because of the high cost of the enzyme purification process, this therapy is subject to serious economical constraints.

STRATEGIES FOR GENE THERAPY

Rationale of the approach

The partial success of BMT, which can only be offered to patients with HLA-matched donors, and the economical obstacles associated with enzyme therapy, have stimulated the search for gene therapy approaches. As in the other therapeutic interventions, the goal is to provide tissues with minimal enzyme levels in order to avoid pathological lysosomal storage. Different strategies must be designed according to the nature of the enzyme. A soluble lysosomal enzyme can be distributed to tissues from autologous cells engineered to secrete it into the blood stream. In the case of membrane-associated or membrane-bound enzymes, gene transfer will have to be targeted to the most affected cells.

The cDNAs for nearly 20 human enzymes involved in LSD have been cloned (Table). Some of them have been transfected into COS or CHO cells to overproduce an active enzyme. Some of these studies also demonstrated that the enzyme was secreted in culture medium and that it could be internalized by deficient cells to restore a normal level of enzyme activity. Normal cDNAs have also been introduced in vitro into deficient cells using retroviral vectors and shown to complement the biochemical and phenotypic defect.[42–49]

Gene transfer into hematopoietic cells

Gene transfer into hematopoietic cells can be performed to complement a deficiency affecting the hematopoietic elements themselves, as in the monocyte/macrophage lineage in Gaucher or Niemann-Pick disease, or to reduce lysosomal storage in non-hematopoietic tissues. In this case the stored substrate can be degraded both by the scavenging activity of infiltrating macrophages derived from corrected stem cells and by other cells that have internalized the enzyme secreted by surrounding geneti-

cally modified cells. Recent data also suggest that reduction of storage may also result from cell-to-cell transfer of the lysosomal enzyme.[50,51]

Efficient procedures for retrovirus-mediated gene transfer into hematopoietic stem cells have been developed in the mouse. Donor bone marrow cells are infected in vitro in the presence of fibroblasts producing the retroviral vector and used to reconstitute lethally irradiated syngeneic recipients. If gene transfer occurs into a stem cell with long-term reconstituting capacity, it may be permanently amplified in the peripheral blood differentiated cell population. Hematopoietic chimeras stably expressing a foreign gene in a majority of peripheral cells from all lineages have been obtained.[52]

Several investigators have used retroviral vectors expressing the human glucocerebrosidase cDNA under the control of the viral LTR to demonstrate efficient transduction into murine long-term repopulating marrow cells. Analysis of long-term reconstituted mice (up to 8 months after transplantation) demonstrated the presence of the provirus in bone marrow, spleen and thymus). When bone marrow cells from these animals were transplanted into secondary recipients, the provirus was again detected in various hematopoietic lineages up to 4 months after transplantation. The levels of human glucocerebrosidase activity in bone marrow and spleen macrophages were equal to or greater than the endogenous mouse activity.[53–56] Efficient transduction of the human glucocerebrosidase cDNA was obtained in vitro into a substantial fraction of human hematopoietic progenitor cells from Gaucher patients.[44,57] These studies have encouraged several investigators to plan clinical trials involving gene transfer. However, in the absence of an adequate animal model for Gaucher disease, a therapeutic effect of gene transfer still has to be demonstrated.

A corrective effect of gene transfer into hematopoietic stem cells on lysosomal storage has been shown in 2 studies in MPS VII mice. In the first study, a retroviral vector coding for the rat β-glucuronidase cDNA under the control of a thymidine kinase promoter was used to infect bone marrow cells of two MPS VII mice. The analysis of the treated animals, 6 months after bone marrow transplantation, showed a complete disappearance of lysosomal storage lesions in the liver and spleen.[58] In a second study partial hematopoietic chimeras were obtained using a low irradiation dose conditioning of the recipient animals. Mice with less than 5% hematopoietic cells containing the human β-glucuronidase cDNA under the control of the phosphoglycerate kinase 1 promoter, displayed a complete correction of the liver and spleen, suggesting that small amounts of enzyme delivered locally can be sufficient for correction.[59] This observation is hopeful for clinical application in man,

since the current available technology in humans does not provide more than a few percent of genetically-modified cells.

Enzyme delivery into the whole organism by genetically modified cells

In LSD involving a secreted enzyme, any cell type could be chosen as a source, provided that efficient methods for ex vivo gene transfer and stable reimplantation exist. Fibroblasts can be easily obtained from skin biopsies, grown in culture and infected with retroviral vectors. The inclusion of fibroblasts into collagen lattices has been shown to result in the formation of transplantable dermis equivalent.[60] The implantation of these lattices into the peritoneal cavity, mixed with bFGF-coated polytetrafluoroethylene (PTFE) fibers was shown to lead to the rapid formation of individualized neo-organs in which the genetically modified fibroblasts are metabolically active for months. A dense vascularisation of the implants brought the enzyme-secreting fibroblasts in permanent contact with the mesenteric circulation.[61] This procedure has been used to secrete human β-glucuronidase in MPS VII mice after retroviral mediated transfer of the human cDNA into skin primary fibroblasts. The implantation into MPS VII mice of lattices containing fibroblasts secreting the human enzyme was followed by a rapid disappearance of lysosomal storage lesions in the liver and the spleen. Human β-glucuronidase activity was found in the liver, spleen, lung, brain, kidney, heart and bone marrow of the implanted animals.[62] These experiments have shown that engineered fibroblasts, if reimplanted in a suitable environment can provide long-term therapeutic levels of enzyme in an MPS model. The cure was not complete however in the animals which displayed severe skeletal abnormalities when they were treated at the age of 6 to 8 weeks.

Experiments are in progress to test whether implanting enzyme-secreting fibroblasts within the first days of life could facilitate the enzyme access to the developing bones and joints and to the central nervous system.

In the perspective of a clinical trial, the procedure has been scaled up in normal dogs. During follow-up of one year uptake of human β-glucuronidase secreted by neo-organs was demonstrated in liver biopsies, in which the canine enzyme was heat-inactivated (P Moullier, unpublished results).

The skeletal muscle has been proposed as a convenient organ for a systemic delivery of therapeutic proteins.[63] Myoblasts have been isolated from MPS VII dog skeletal muscle, grown in culture and infected with a rat β-glucuronidase cDNA-containing retroviral vector. Enzyme expression was documented in both myoblasts and myotubes.[64]

Myoblasts from adult MPS VII mice were also isolated and infected with a retroviral vector coding for human β-glucuronidase. These cells were then injected in MPS VII mice, following muscle injury. The genetically-modified cells were found to efficiently participate to the constitution of regenerated muscle fiber. However, despite an efficient in vitro secretion of the enzyme, only trace amounts of activity were found in the liver and spleen of the treated animals.[65] This suggested that β-glucuronidase was blocked before it could access the blood stream, possibly at the level the muscle basal membrane or immediately reinternalized through binding to M6PRs which are highly expressed in muscle cells.

The liver occupies a strategic position as a provider of proteins into the blood stream. Retrovirus-mediated gene transfer in situ into the liver has been described in mice, rats and dogs.[66,67]Attempts at transferring the β-glucuronidase cDNA into the liver of MPS VII dogs are currently being made. The first results indicate that the fraction of hepatocyte which can be modified by this procedure may be too small to provide therapeutic enzyme levels.

Enzyme delivery to the central nervous system

It is unlikely that a soluble lysosomal enzyme delivered into the serum will cross the blood-brain barrier under normal conditions.[68] The β-glucuronidase found in the brain of MPS VII mice implanted with fibroblasts secreting the enzyme may correspond to enzyme molecules absorbed by monocytes in the periphery and transported across the barrier.[62] In this case, however, the small amount of enzyme found in this tissue may be too low to obtain a correction of the lysosomal storage lesions.

Crossing of the blood-brain barrier could be achieved by coupling the soluble enzyme to an antibody or a ligand recognized by a receptor present on the surface of endothelial cells. It was shown that when NGF was coupled to an antibody against the transferrin receptor, it could cross the blood-brain barrier after peripheral injection in rats.[69] However, in the case of lysosomal enzymes, fusion molecules may loose their catalytic activity or their ability to be recognized by the M6P receptor. Whether these large molecules can be efficiently transported across the endothelial cells also remains to be demonstrated.

Another possible problem may be that, even if the soluble enzyme can cross the blood-brain barrier, it may not be taken up by the cells that need to be corrected. Indeed, delivery of hexosaminadase A to the brain of GM2 gangliosidosis cats, by reversible blood-brain barrier permeabilization lead to a significative concentration of the enzyme in the brain but no detectable uptake by neurons which are the affected

cells. Targeting of neurons was obtained in vitro only after coupling of hexosaminidase A via disulfide linkage to the atoxic fragment C of tetanus toxin.[70]

An alternative solution would be to install intracerebral implants of genetically-modified fibroblasts and myoblasts.[71,72] In the case of LSD however, enzyme delivery throughout the brain is needed and the modified cells should be able to migrate after implantation. Genetically-modified astroglial (O2A) progenitors can be used to assess the capacity of a limited number of cells scattered in the brain to eliminate lysosomal storage. However, the difficulty to access the target cell for ex vivo gene transfer makes this procedure of little therapeutic relevance. Multipotent immortalized neural progenitor cell lines with high migration capacity have been described in the mouse[73] and used to obtain long-term diffuse engraftment in the brain of newborn MPS VII animals after the transfer of the β-glucuronidase cDNA (J Wolfe, personal communication). The availability of such cells in humans could be of genuine interest for the treatment of CNS lesions in LSD.

Direct gene transfer into the CNS is feasible with herpesvirus or adenovirus vectors. A recombinant HSV-1 virus encoding for the rat β-glucuronidase was used to infect MPS VII mice by corneal inoculation. After several weeks, few positive neurons were detected by histochemical staining in the trigeminal ganglia and brain stem of treated mice.[74] The disappearance of lysosomal storage in or around the positive cells was not studied. Although this has not been tested in LSD models yet, a more potent gene transfer can be obtained with adenovirus, by stereotactic injection of vector particles in the brain tissue or in the ventricular space. This second approach leads to the infection of the ependymal cells lining the ventricule and can be used to secrete a protein in the CSF. In LSD, this could directly reduce the levels of undegraded molecules in the CSF and might help enzyme diffusion to large areas of the brain.

Perspectives for clinical trials

Four gene therapy trials have already been approved for the treatment of Gaucher disease by retroviral-mediated transfer of the human glucocerebrosidase cDNA into hematopoietic stem cells.[75] Human CD34+ cells will be purified from G-CSF-mobilized peripheral blood stem cells or from bone marrow and transduced with retrovirus containing media. The retroviral vectors used express the human glucocerebrosidase cDNA under the control of the viral LTR. The transduced cells will then be infused into the patient. If peripheral blood stem cells are used this procedure can be repeated several times while if bone marrow is used, only one treatment will be done. The aims of these trials are: (i) to

examine the safety and the efficiency of transducing the human glucocerebrosidase into CD34 cells by retrovirus-mediated gene transfer; (ii) to determine the extent of long-term persistence of transduced cells in patients; (iii) to investigate whether the enzyme is expressed efficiently enough to improve the patient conditions. For safety reasons, early trials do not include myeloablative conditioning treatment. It is not known whether a therapeutic effect can be obtained in such conditions, since low levels of engraftment of genetically-modified cells are expected.

Regarding MPS, in vivo gene transfer data have been obtained with MPS VII animals and it would seem logical to consider patients with Sly syndrome as the first candidates for a gene therapy trial. However, these patients are exceedingly rare, with less than 20 known cases of live birth. Hurler disease is one of the more frequent MPS. A genotype/phenotype correlation has begun to be established and pre or perinatal diagnosis is feasible. The mechanisms of synthesis, processing, secretion and uptake of β-glucuronidase and α-L-iduronidase are similar, and it is likely that most of the gene transfer data obtained in MPS VII animals can be extrapolated to MPS I. Analysis of Nude mice implanted with neo-organs secreting the human α-L-iduronidase indicates that the enzyme is internalized in the liver and the spleen as efficiently as β-glucuronidase (A Salvetti, unpublished results). The therapeutic efficacy of the gene therapy approaches defined in MPS VII models can also be tested in MPS I dogs.[12] Two types of intervention on MPS I patients could be proposed in the near future, involving retrovirus-mediated gene transfer to either hematopoietic ($CD34^+$) cells or to skin fibroblasts reimplanted into the peritoneal cavity. The graft of autologous skin fibroblast secreting α-L-iduronidase from vascularised neo-organs could be performed using a minimally invasive surgical procedure. Initial trials will have to assess the feasibility of the procedure, its tolerance by the patient, the efficiency and duration of enzyme secretion and the effect on the course of the disease.

More clinical trials are likely to be organized within the next few years for the treatment of other LSD, and Niemann-Pick A and B or metachromatic leucodystrophy are likely candidates. However, the multiplication of clinical trials will critically depend on the issue of the early ones, which therefore have to be conducted very rigorously. As clinical applications will progress, it will remain essential to perform careful experiments in animal models. The uncommon wealth of animals affected with these diseases provides a unique opportunity to base gene therapy trials on a solid collection of scientific and preclinical data.

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Myoblast-based gene therapies

T A Partridge[1] and K E Davies[2]

[1]MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK and [2] Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, UK

Recent identification of the genetic causes of several neuromuscular disorders has aroused interest in gene therapy in skeletal muscle. The genetic constitution of skeletal muscle can be altered by a number of means. Myoblasts can be used to introduce new genes, endogenous or exogenous, into muscle fibres during growth and repair. DNA expression-plasmids can be directly transfected into a small proportion of muscle fibres, showing persistent expression despite their lack of genomic integration. Recombinant replication deficient adenoviruses are efficient vectors into myoblasts and developing muscle fibres; again, the introduced constructs show long-term episomal persistence and expression. By contrast, recombinant replication deficient retroviruses efficiently introduce constructs into the genomes of dividing myoblasts which subsequently fuse into muscle fibres. None of the available methods provides a practical solution for therapy of genetic muscle diseases but might be useful for inducing synthesis of therapeutic non-muscle proteins by skeletal muscle

SPECIAL INTEREST OF SKELETAL MUSCLE AS A TARGET FOR GENE THERAPY

To one unfamiliar with the field, general interest in skeletal muscle as a target for gene therapy might come as a surprise since it has no obvious single quality, apart from its abundance, to commend it. For explanation, one must look to a conjunction of individual factors, including properties of the mature tissue, its developmental biology, its ease of tissue culture and perhaps especially to historical events – such as the elucidation, over the past few years, of the genetic basis of primary

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